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**Stimulatory autoantibodies to PDGFR in systemic sclerosis: What functional
autoimmunity could learn from receptor biology**

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Introduction

The molecular mechanisms leading to tissue fibrosis in patients with systemic sclerosis (SSc) are still incompletely understood. However, accumulating evidence suggests that profibrotic cytokines are a major driving force for the activation of fibroblasts and fibroblast-like cells, resulting in an increased release of extracellular matrix proteins. These profibrotic cytokines are often upregulated throughout the course of the disease, which could explain the ongoing activation of fibroblasts in affected tissues. Platelet derived growth factors (PDGFs) are among the best characterized profibrotic cytokines in SSc. This editorial summarizes evidence for a key role of PDGFs in the pathogenesis of SSc and tries to find explanations for the controversial results on the presence of stimulating autoantibodies against PDGF receptors (PDGFR) in SSc.

PDGF and PDGFR in systemic sclerosis

In mammals, the family of PDGFs consists of four different members, PDGF-A, PDGF-B, PDGF-C and PDGF-D, which are encoded by four distinct genes (1). Dimerization of these single PDGF chains is required for biological activity. In addition to homodimers of all four single chains (PDGF-AA, PDGF-BB, PDGF-CC and PDGF-DD), heterodimers of PDGF-A and PDGF-B have been identified (PDGF-AB). PDGFs act via two receptor tyrosine kinases, PDGFR α and PDGFR β (1). PDGFR α is activated by PDGF-AA, PDGF-AB and PDGF-CC, whereas PDGF-BB, PDGF-AB and probably also PDGF-DD bind to PDGFR β .

PDGF-A and PDGF-B isoforms play key-roles in the pathogenesis of SSc and other fibrotic disorders (2). Although PDGF-C and PDGF-D have also linked to fibrosis, their significance for the pathogenesis of SSc has not yet been investigated. PDGF and PDGFRs are overexpressed in SSc. Gay et al. demonstrated an increased immunostaining for PDGF-B in endothelial cells and infiltrating macrophages in lesional skin of SSc patients (3). Elevated

levels of PDGF activity were also found in the blood and in bronchoalveolar fluid of SSc patients (4, 5). Moreover, Klareskog and coworkers showed that PDGFR β was strongly expressed in dermal vessels and in fibroblasts of SSc patients, but not in healthy individuals (6). Interestingly, overexpression of PDGFR β was not restricted to lesional skin, but also observed in clinically non-involved skin.

The increased production of PDGF in SSc might be driven by a combination of other cytokines and mediators. Interleukin-1 α seems to be a potent stimulator of the expression of PDGF. In contrast to fibroblasts from healthy volunteers, SSc fibroblasts constitutively express interleukin-1 α , which in turn stimulates the release of PDGF-AA and increases proliferation (7). Dysregulation of TGF- β signaling cascades might also activate PDGF signaling in SSc. TGF- β 1 stimulates the release of PDGF-AA and increase the expression of PDGFR α in SSc fibroblasts. In normal fibroblasts, stimulation with TGF- β 1 does not affect the expression of PDGFR α and higher concentrations of TGF- β 1 decrease the expression of PDGF-AA (8-10). Additionally, the serine protease thrombin and interleukin 13 are potent inducers of PDGF-AA and PDGFR α and might stimulate PDGF signaling in SSc (11, 12).

PDGF isoforms contribute to the pathogenesis of fibrotic diseases primarily by their stimulatory effects on fibroblasts. PDGF promotes not only chemotaxis and stimulates proliferation, but also enhances myofibroblast differentiation, increases collagen production and promotes fibroblast adhesion of cultured SSc fibroblasts in vitro (reviewed in (2)). Pro-fibrotic effects of PDGF were also observed in different animal models. Intratracheal instillation of PDGF-BB induced proliferation of mesenchymal cells and stimulated the accumulation of collagen in rats (13). Furthermore, overexpression of PDGF-B in mice under control of the surfactant C promoter lead to pulmonary fibrosis accompanied by emphysema-like changes (14). Consistently, overexpression of PDGF-BB by an adenoviral approach also

resulted in mild pulmonary fibrosis (15). Overexpression of PDGF A or B chains in other organs than the lungs also resulted in tissue fibrosis.

Indirect evidence for the importance of PDGF comes from therapeutic approaches targeting PDGF signaling. Selective blockade of PDGF signaling either by antibodies or by small molecule inhibitors such as SU9518 exerts anti-fibrotic effects in different models of experimental fibrosis (16, 17). In addition, the combined inhibition of PDGFRs and c-abl by tyrosine kinase inhibitors such as imatinib, dasatinib or nilotinib prevents the development of fibrosis in different preclinical models (18-21).

Although PDGF is sufficient to induce fibrotic changes in vivo, the fibrotic changes induced by PDGF alone were mild to moderate in all studies. Furthermore, the fibrotic changes induced by PDGF alone might be transient. Yi et al observed that the collagen deposition upon application of recombinant PDGF-BB resolved completely within days after cessation of the PDGF application (13). However, the pro-fibrotic effects of PDGF and TGF- β might augment each other in SSc leading to persistent fibrosis. Consistent with this hypothesis, the combined overexpression of PDGF-B and TGF- β 1 resulted in strong fibroproliferative response with prominent fibroblast proliferation and collagen accumulation that was significantly more pronounced than that induced by PDGF-B alone (15).

Evidence for the role of B cells in SSc

B cells play a central role in many immune responses. B cells are precursors of antibody-secreting plasma cells and are thus involved in the production of autoantibodies in SSc. In addition, B cells function as antigen presenting cells, release various cytokines and influence T cell differentiation and dendritic cell functions (22, 23). Multiple lines of evidence suggest that B cells numbers and functions are altered in SSc. Total B cell counts are elevated in SSc with increased counts of naïve B cells, but decreased numbers of memory B cells and

differentiating early plasma cells (23). B cells and plasma cells are also found in inflammatory infiltrates in affected tissues of SSc patients such as the skin and the lungs (24, 25). Consistent with these findings, a prominent B cell signature has been found by microarray in skin biopsies of SSc patients with significant overexpression of immunoglobulins and other genes related to B cells (26).

Naïve B cells and memory B cells from SSc patients are both activated in SSc and express increased levels of CD19 (27). Although the levels of CD19 were elevated by only 20% compared to controls, this modest upregulation might already predispose to autoimmunity. Sato et al. demonstrated that TG-4 mice, which overexpress CD19 to a similar extent as SSc patients, have significantly increased levels of autoantibodies including anti-topoisomerase antibodies (28). The central role of CD19 for the production of autoantibodies was further outlined by studies on mice deficient for CD19 (29). Hypergammaglobulinemia, autoantibody production and hypodermal thickening are decreased in tight skin-1 mice deficient for CD19 compared to tight skin-1 mice expressing normal levels of CD19 (29). CD19 deficiency ameliorates fibrosis also in the mouse model of bleomycin induced fibrosis (30). However, TG-4 mice did not develop fibrosis spontaneously, suggesting that overexpression of CD19 and B cell activation influence the outcome of fibrosis in predisposed mice, but are alone not sufficient to induce fibrosis. Clinical studies with B-cell depleting medications such as rituximab are under way in patients with SSc.

Stimulatory autoantibodies to PDGFR in SSc: What is the truth?

A novel link between B-cell mediated autoantibody production and upregulated PDGFR signalling in SSc was provided by the landmark paper from Baroni et al (31). In this paper, the authors showed the presence of stimulatory antibodies to PDGFR in all patients with SSc using a bioassay consisting of mouse-embryonic fibroblasts with or without PDGFR subunits.

Isolated IgGs from patients showed functional agonistic activity by inducing tyrosine phosphorylation and the production of reactive oxygen species. In addition, a number of control experiments including preincubation of IgGs with recombinant PDGFR and preincubation of mouse embryonic fibroblasts with PDGFR tyrosine kinase inhibitors abolished the effects of the stimulatory autoantibodies. Moreover, isolated IgGs from SSc patients induced myofibroblast conversion and type I collagen expression in normal human fibroblasts. Stimulatory autoantibodies to PDGFR were later also found for patients with extensive chronic graft versus host disease as another fibrotic disease (32), but were not seen in patients with primary Raynaud's phenomenon and other autoimmune rheumatic diseases.

These exciting results now appear to be difficult to reproduce in other SSc populations. In this issue of *Arthritis & Rheumatism*, two articles are published that failed to detect stimulatory autoantibodies in the serum of patients with SSc. Loizos et al (33) used electrochemiluminescence binding assays to detect binding of purified IgG to PDGFR. Binding activity to PDGFR was found in 33% of SSc patients, but was not specific for SSc, and was also detected in 34% of healthy controls. In addition, antibodies binding to PDGFR did not reveal agonistic activity, when tested in porcine aortic endothelial cells stably expressing human PDGFR α . In the second paper by Classen et al (34), the authors generated a 32 D mouse cell line transfected with human PDGFR α and β to assess the agonistic activity of purified IgGs to PDGFR. In addition, mouse-embryonic fibroblasts with or without PDGFR α as in the paper by Baroni et al were used. With these assays, the stimulatory activity of IgG purified from SSc patients was overall weak and not different from IgGs purified from controls (healthy controls and patients with systemic lupus erythematosus). These results are consistent with a recently published paper, which did not reveal differences in the levels of PDGFR antibodies of SSc patients and controls using an ELISA system, although no functional assays were performed to analyze the agonistic activity of these antibodies (35).

How can these controversial results be reconciled? Results from Baroni et al implicate that the PDGFR epitope needs to be in its native configuration to be recognized by the stimulatory autoantibody. This native configuration awaits identification. Thus, differences in the conformation of PDGFRs used in the assays could account for at least some of the controversial results. Indeed, the biology of PDGFR signaling with respect to its conformation is complex. In the absence of ligands, PDGFRs are either monomeric or dimeric, but are inactive. Regulation of the catalytic activity in tyrosine kinase receptors such as PDGFR is determined by a key-structural element, the so-called activation segment/loop in the tyrosine kinase domain (36). In general, there is evidence to support the existence of ligand selective receptor conformations. Thus, not all agonists necessarily lead to the same receptor activation state. In addition, whether ligand binding leads to dimerization of monomeric receptors or conversion of an inactive non-signalling receptor dimer to an active signalling dimer is still an area of investigation (37). Moreover, in the case for PDGF-R β , dimerization is necessary, but not always sufficient for tyrosine kinase activation (38). There are also differences between active receptors induced by agonists and active receptors which occur constitutively without ligand activation. These phenomena are for instance well known for G-protein receptors (39). This is of interest, as the concept of functional autoimmunity comes from G-protein receptor biology in Graves disease, in which stimulatory autoantibody to the thyroid-stimulating hormone receptor (TSHR) play a major role in the pathogenesis (40). Thus, a plethora of factors related to the conformation of PDGFR can account for the different results of studies on agonistic PDGF-autoantibodies. The fact that Claasen et al used a similar cellular system as Baroni et al for some of their functional experiments does not exclude this possibility, as there were slight differences in handling of these cellular systems. In addition, the stimulatory effects of SSc IgG were present, although mild and not significantly discernible from control effects. However, it would have been interesting to see whether the blockade of PDGF-R

would have abolished SSc-specific and not control IgG-related read-outs, considering well described Fc-related IgG effects on some cellular processes. On the other hand, it remains to be shown, whether the obviously very specific confirmation of PDGFR required for the effects of stimulatory autoantibodies is biologically relevant.

What should we learn from these studies with heterogeneous results? In order to enhance our understanding we should learn from other fields with a long-standing experience in stimulatory autoantibodies. In particular, the studies on TSHR provide important lessons. Beside antibodies that activate TSHR, there are also antagonizing TSHR autoantibodies, as well as neutral antibodies that do not affect TSHR function. The TSHR stimulating antibodies of Graves disease are similarly dependent on the conformation of the TSHR and have no interaction with nonglycosylated or reduced receptors, while shed TSHR ectodomains may act as immune stimulants or antibody decoys. It took decades to develop reliable clinical assays for the detection of TSHR antibodies.

In any case, there is a “lengthy to do list” in order to assess the role of stimulatory autoantibodies against PDGFR in the pathogenesis of SSc. Search for epitopes and generation of the synthetic polyclonal and monoclonal antibodies certainly belong to the priority tasks. At this stage of the development in the field, the first step in the right direction would be the development of a standardized screening procedure for functionally active human anti-PDGFR antibodies. This procedure would require consensus on the cell system presenting the target receptor in its natural conformation and the conditions to use it, in order to provide unequivocal interpretation of future results.

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